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Review

# Gaseous mediators in resolution of inflammation

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ABSTRACT

There are numerous gaseous substances that can act as signaling molecules, but the best characterized of these are nitric oxide, hydrogen sulfide and carbon monoxide. Each has been shown to play important roles in many physiological and pathophysiological processes. This article is focused on the effects of these gasotransmitters in the context of inflammation. There is considerable overlap in the actions of nitric oxide, hydrogen sulfide and carbon monoxide with respect to inflammation, and these mediators appear to act primarily as anti-inflammatory substances, promoting resolution of inflammatory processes. They also have protective and pro-healing effects in some tissues, such as the gastrointestinal tract and lung. Over the past two decades, significant progress has been made in the development of novel anti-inflammatory and cytoprotective drugs that release of one or more of these gaseous mediators.


## 1. Introduction

Since the identification of nitric oxide (NO) as the substance accounting for the actions previously attributed to 'endothelium-derived relaxing factor' [1], there has been a burst of research into several gaseous mediators. NO, hydrogen sulfide ($H_2S$) and carbon monoxide (CO) have been the most studied gaseous mediators over the past three decades, and attempts have been made to develop novel anti-inflammatory drugs based on delivery of one or more of these gaseous mediators [2]. All three of these mediators have very low molecular weights, short half-lives, and can freely diffuse across membranes. They do not have specific receptors, per se, but can interact with a range of proteins and genes to produce a wide range of effects. Particularly in the case of NO and $H_2S$, suppression of synthesis can profoundly alter cell and tissue function.

The potential for the actions of these mediators to be exploited in the design of therapeutics is considerable. Of course, drugs that release NO have been in use for over a century (e.g., nitroglycerin). Development of drugs based on stimulation or suppression of $H_2S$ or CO production has occurred mainly in the past decade. In this article, we review some of the key actions of these gaseous mediators with respect to their impact on inflammatory processes. We also review some of the drugs in development that are aim to exploit the anti-inflammatory actions of these mediators.

## 2. Nitric oxide

### 2.1. The importance of L-arginine-nitric oxide pathway

The acute inflammatory response is a self-limiting process that normally results in restoration of tissue homeostasis. Persistent inflammatory stimuli or deregulation of mechanisms of the resolution phase results in chronic inflammation, recognized to be a key underlying factor in the progression of a range of diseases, including atherosclerosis, arthritis, cancer and chronic neurodegenerative diseases. Alterations in NO synthesis by endogenous systems can influence these inflammatory processes.

The inorganic free radical, NO was first identified as an endothelium-derived endogenous messenger responsible for the regulation of vascular tone [3,4]. However, since then it has become clear that NO is the signaling molecule responsible for several diverse physiological and pathophysiological processes. NO is produced by three different forms of NOS, namely nNOS (NOS1), eNOS (NOS3), and iNOS (NOS2) [5].

A rather simple, not fully correct but traditionally useful classification discriminates inducible versus constitutively expressed NOS isoforms, which approximates low versus high production rates of endogenously generated NO [5,6]. Thus, it has been generally assumed that nNOS and eNOS are critical for a normal physiology, whereas iNOS is associated with injury. NOS isoforms not

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only produce NO, the primary reaction product, but also a number of species resulting from oxidation, reduction, or adduction of NO in physiological milieus, thereby generating various clinical species such as, S-nitrosothiols, peroxynitrite (ONOO—), and transition metal adducts [7]. The classical pathway by which NO exerts many of its actions is via activation of the enzyme soluble guanylate cyclase (sGC) [8] and resultant conversion of guanosine 5′-triphosphate (GTP) to the second messenger 3′,5′-cyclic guanosine monophosphate (cGMP) [9]. However, several studies have established that NO can also act via cGMP-independent pathways in various systems, particularly during the inhibition of platelet aggregation and regulation of inflammatory cell apoptosis [10–13]. In addition, NO modulates transcription/translation indirectly by affecting signaling pathways, such as mitogen activated protein kinases, G-proteins, the Ras pathway, glyceraldehyde dehydrogenase or phosphatidylinositol-3 kinase (PI3K) [14].

### 2.2. NO apoptosis and resolution of inflammation

Progression of inflammatory conditions depends not only upon the recruitment and activation of inflammatory cells but also upon their subsequent removal from the inflammatory milieu. Apoptosis, or programmed cell death, can be considered as a "removal" mechanism, leading to resolution of inflammation, characterized by a series of morphological and biochemical features [15]. In inflammation-based diseases apoptosis is a fundamental process regulating inflammatory cell survival and it is critically involved in ensuring the successful resolution of an inflammatory response [15]. Dysregulation of apoptosis and phagocytic clearance mechanisms can have drastic consequences on resolution of inflammatory processes. Hence, apoptosis represents a mechanism to remove potentially damaging pro-inflammatory cells from the site of inflammation and is therefore critical to the successful resolution of inflammation. During this process, activated inflammatory cells generate reactive oxygen and nitrogen species, including NO. In this context it is of particular interest the ability of NO to regulate apoptosis of inflammatory cells [16]. What makes complex to evaluate the involvement of NO in the resolution phase is that NO has both pro-apoptotic and anti-apoptotic properties [17–19]. However, it is known that lower concentrations of NO produced by the eNOS and nNOS are cytoprotective, whilst supraphysiological concentrations produced by the iNOS trigger cell death. These apparent opposite effects are explained, at least in part, by the free radical nature of NO and hence its chemical interaction with other radicals present in the milieu to form various NO-related species in vivo. The pro- or anti-apoptotic effects of NO may thus be critically governed by the specific NO-related species generated. An example is represented by the ONOO— species that could account for the apoptotic resolving process [20]. However, the precise role of ONOO— in inflammatory cell apoptosis is still not clearly defined. Thus, it is intuitive that the ability of NO to induce apoptosis is particularly relevant during the resolution phase of inflammation. Several studies have demonstrated that activated macrophages infiltrating murine tumors induce apoptosis via a NO-dependent pathway in both activated anti-tumor T cells and in the tumor cells themselves [21,22]. Thus, it appears that macrophages induce apoptosis of nearby cells through NO that in turn enhances the clearance of apoptotic cells thereby promoting the resolution phase of inflammation.

It is widely accepted that inflammation drives development of some cancers that adapt to and use the oxidant-rich microenvironment [23,24]. This latter phenomenon provides a persistent and self-perpetuating oxidative stress composed of both reactive nitrogen species and reactive oxygen species [25]. Among the critical oxidant sources NO plays a major role in oxidative stress in melanoma and other cancers [25–29].

Also in cancer the effect of NO is dependent upon the concentration since it has been shown that angiogenesis, proliferation and metastasis can normally be stimulated by lower levels of NO (<100 nM), while higher concentrations of NO (>400–500 nM) promote cytotoxicity and cell apoptosis [30,31]. Thus, NO donors have been proposed as a novel therapy to various cancers [32,33]. The most interesting results have been obtained by the use of nitric oxide-releasing non-steroidal anti-inflammatory drugs (NO-NSAIDs). In fact, several studies have found a link between NSAIDs use and decreased risk of colorectal cancer [34–36], suggesting a possible chemopreventive role. NO-NSAIDs have been shown to be more effective in the inhibition of cancer cell growth and metastasis than the parent drug alone [33,37].

Atherosclerosis is another diseases where the concept of resolution of inflammation can be applied with regard to NO. It is now widely recognized that the inflammatory component of atherosclerosis contributes to plaque formation [38–40]. Plaque growth and development are driven by inflammatory cells, in particular monocytes and macrophages, representing the major driving force. Because apoptotic cells are ingested by phagocytes without initiating any further pro-inflammatory response, it has been suggested that apoptosis may represent a mechanism to regress the plaque. Therefore this process triggered by NO contributes to resolution of inflammation [41,42].

In conclusion NO plays a role in the resolution of inflammation but its activity depends upon the concentration of NO in the local environment, the timing of administration or the route of administration, as well as the NOS isoform targeted [43,44].

## 3. Hydrogen sulfide

### 3.1. $H_2S$ as a mediator of inflammation

Kimura and colleagues were the first to identify physiological roles for $H_2S$ through their studies of its actions in the nervous system [45,46]. Several years, later, Wang and colleagues demonstrated the $H_2S$ was a potent endogenous vasorelaxant [47]. Together, these studies stimulated a burst of research into the role of this gaseous mediator in many cells and organ systems [47,48]. Zanardo et al. provided key evidence suggesting a role for $H_2S$ an important endogenous anti-inflammatory and pro-resolution mediator [49]. They reported that $H_2S$ exerted potent inhibitory effects on leukocyte adherence to the vascular endothelium. Using intravital microscopy to examine the mesenteric microcirculation in rats, they demonstrated that administration of $H_2S$ (using donors such as $Na_2S$, NaHS and Lawesson's reagent) markedly and potently suppressed leukocyte adherence of leukocytes to the vascular endothelium and prevented extravasation of leukocytes [49]. They also observed that the inhibition of endogenous $H_2S$ synthesis resulted in a very fast induction of leukocyte adhesion to the vascular endothelium. In models of carrageenan-induced sub-dermal inflammation, $H_2S$ donors were found to suppress leukocyte infiltration and edema formation [49,50]. Inhibition of leukocyte-endothelial adhesion by $H_2S$ is a consequence of suppression of the expression of cell adhesion molecules on both the endothelium (e.g., intercellular adhesion molecule (ICAM)-1 and P-selectin) and on the leukocyte (lymphocyte function-associated antigen (LFA)-1) [49]. Several other groups have also reported inhibitory effects of $H_2S$ on these adhesion molecules [51–54]. Consistent with the findings of Zanardo et al. [49], mice that are heterozygous for the gene for cystathionine β-synthase (CBS), one of the major enzymes for synthesis of $H_2S$, exhibit reduced leukocyte-rolling velocity, increased vascular permeability, and increased numbers of adherent leukocytes in mesenteric venules [55].

In addition to $H_2S$ potently inhibiting leukocyte adhesion to the vascular endothelium, numerous studies have shown that $H_2S$ can directly influence a wide range of leukocyte functions. For example, a recent study demonstrated that $H_2S$ inhibits the activity of the granulocyte enzyme myeloperoxidase (MPO), which had been isolated from humans and rats [56]. MPO catalyzes the conversion of hydrogen peroxide to hypochlorous acid. The latter has powerful bactericidal effects, but also has the capacity to cause significant damage to host tissue. $H_2S$ has been shown to be an avid scavenger other granulocyte-derived cytotoxic substances, including peroxynitrite [57], superoxide anion [58], hypochlorous acid [59] and hydrogen peroxide [60].

Two of the most critical processes in resolution of inflammation are induction of neutrophil apoptosis and stimulation of macrophages to phagocytose apoptotic neutrophils (i.e., driving macrophages to a "M2" phenotype) [61]. An inability to clear neutrophils contributes significantly to the development of chronic inflammation. $H_2S$ promotes neutrophil apoptosis [62]. Dufton et al. [61] demonstrated that application of $H_2S$ to human or murine macrophages increased chemotaxsis of macrophages and the rate of phagocytosis of the bacterium *Escherichia coli*. Both are stimulated by $H_2S$, resulting in a reduction of the accumulation of inflammatory cells [61]. Exposure to $H_2S$ also rendered macrophages hyporesponsive to inflammatory stimuli such as bacterial endotoxin or TNF-α.

Some anti-inflammatory actions of $H_2S$ are mediated by annexin-A1, another pro-resolution mediator [52]. Treatment of isolated human neutrophils with an $H_2S$-releasing agent (NaHS) resulted in translocation of annexin-A1 from the cytosol to the plasma membrane. This is an event that is required for the initiation of annexin-A1 signaling. In studies in mice, treatment with an $H_2S$ donor inhibited IL-1-stimulated granulocyte adhesion and extravasation in mesenteric venules, as well as down-regulating endotoxin-induced expression of COX-2 and inducible NO synthase in macrophages [52]. When similar experiments were performed in annexin-A1-deficient mice, both of these effects were absent. Moreover, in the annexin-1 deficient mice there was increased expression of CBS and cystathionine γ-lyase (CSE) in a range of tissues, further demonstrating a link between annexin-A1 and $H_2S$ in promoting resolution of inflammation [52].

Many key mediators of inflammation are regulated to some degree by $H_2S$. This represents another means through which $H_2S$ regulates processes such as leukocyte adhesion and emigration. In the GI tract, for example, $H_2S$ has been shown to play a crucial role in promoting the constitutive expression of cyclooxygenase (COX)-2 and of prostaglandin synthesis [63,64]. This is important for maintenance of mucosal integrity, but also for rapid responses to injury. In this context, $H_2S$ acts to maintain physiological expression of COX-2. However, in other circumstances, such as when there is chronic inflammation of the tissue, $H_2S$ can dampen inflammation-associated up-regulation of COX-2 expression and of a number of other pro-inflammatory cytokines, thereby contributing to resolution of inflammation [65]. For example, $H_2S$ has been shown to down-regulate the expression of a range of pro-inflammatory cytokines (e.g., IL-1β, tumor necrosis factor (TNF)α, interferon (IFN)γ, IL-12, IL-23, etc.). In contrast, $H_2S$ does not suppress, and in some circumstances elevates, the expression of IL-10, a powerful anti-inflammatory cytokine [65,66]. The ability of $H_2S$ to affect the expression of so many pro-inflammatory cytokines is likely due to its modulating effects on nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activity [67]. As described in more detail below, IL-10 also plays an important role in the regulation of $H_2S$ synthesis [68].

In addition to its role in inflammation, COX-2-derived prostaglandin synthesis is an essential element of GI mucosal defence and repair [63], and its modulation by $H_2S$ is very important. For example, if healthy rats are treated with pharmacological inhibitors of $H_2S$ synthesis, there is a significant reduction of the expression of COX-2, a concomitant decrease in mucosal prostaglandin $E_2$ synthesis, and the development of mucosal inflammation in the absence of any detectable tissue injury [69,70]. On the other hand, administration of an $H_2S$ donor (diallyl disulfide) resulted in a marked, dose-dependent elevation of COX activity in the small intestine, as well as a reduction in mucosal inflammation [71].

### 3.2. $H_2S$ in promotion of repair

In addition to its anti-inflammatory effects, there is considerable evidence that $H_2S$ contributes significantly to promoting repair to tissue injury and restoration of tissue function. Some of the earliest studies of the role of $H_2S$ in resolution of tissue injury utilized models of inflammation and injury in the GI tract [70,72,73]. For example, administration of $H_2S$ donors or with L-cysteine (precursor for $H_2S$ synthesis) markedly accelerated healing of ulcers in the stomach of rats, while suppression of $H_2S$ synthesis resulted in a marked delay in healing [73]. Endogenous $H_2S$ synthesis was markedly elevated, as was the expression of two of the key enzymes for $H_2S$ synthesis CSE and CBS, particularly along the ulcer margin, where re-epithelialization is most active [73]. This is consistent with the powerful angiogenic effects of $H_2S$ [74]. Interestingly, non-steroidal inflammatory drugs (NSAIDs) are know to impair gastric ulcer healing, but treatment of mice that had gastric ulcers with an $H_2S$-releasing NSAID (ATB-346) resulted in a marked acceleration of healing [75]. This occurred despite the compound markedly suppressing gastric prostaglandin synthesis, which is believed to be responsible for COX-2-driven angiogenesis and repair. Thus, $H_2S$ appears to have additional pro-healing mechanisms to those mediated via induction of COX-2 expression.

### 3.3. Rapid $H_2S$ response to injury

$H_2S$ acts as a 'rescue molecule' in many circumstances, helping to preserve tissue integrity and function while repair occurs. It addition to the rapid up-regulation of the enzymes that produce $H_2S$ from L-cysteine, which drive tissue repair, $H_2S$ can markedly reduce tissue injury associated with hypoxia/anoxia by preserving mitochondrial function. $H_2S$ can 'drive' mitochondrial respiration, and the generation of adenosine triphosphate, particularly in circumstances where oxygen levels are very low, such as during ischemia [76–78]. There is substantial evidence from studies of the GI tract demonstrating this 'rescue' role of $H_2S$. Indeed, the epithelial cells of the GI tract have been reported to be the most efficient at using $H_2S$ to drive mitochondrial ATP production [76–78].

As mentioned bove, when colonic inflammation is induced in animal models, there is a very rapid and profound increase in $H_2S$ synthesis, and up-regulation of the key enzymes for $H_2S$ synthesis [70]. These increases in $H_2S$ synthesis and enzyme expression occur specifically at the sites of tissue injury – there is no change in the immediately adjacent inflamed, but not damaged tissue [79]. Moreover, there is a down-regulation of oxidation of $H_2S$ at these same sites of damage [79]. Of course, the net result of the elevated synthesis and decreased degradation of $H_2S$ would be greater local concentrations of this mediator, which drives tissue repair. Administration of inhibitors of $H_2S$ synthesis in models of colitis results in a dramatic increase in the severity of disease; indeed, in some studies, colonic perforation and death occurred [70]. Suppression of $H_2S$ synthesis in rats with colitis was also associated with a marked thickening of the wall of the colon, mainly attributed to hyperplasia of the layers of muscle. Administration of $H_2S$-releasing drugs, such as NaHS or Lawesson's reagent, promotes resolution of colitis [66,70]. These drugs also reduced the thickening of colonic

muscle, and reduced the levels of TNFα in colonic tissue [70]. Similar results have also been demonstrated in a model of intestinal ulceration [71], further highlighting the ability of $H_2S$ to promote tissue repair.

Another demonstration of a clinically relevant role for $H_2S$ in driving resolution of injury and inflammation in the digestive tract involved the induction of hyperhomocysteinemia, induced by feeding a vitamin B-deficient diet to rats [68]. Approximately one-third of patients with inflammatory bowel disease (IBD) exhibit vitamin B deficiency, which can lead to hyperhomocysteinemia, and there is clear evidence that this condition significantly worsens the severity of IBD [80–82]. In rats with diet-induced hyperhomocysteinemia, we observed that there was significant impairment of colonic $H_2S$ synthesis, and an absence of the up-regulation of CSE expression typically seen in the colon when it is inflamed [70]. These changes were observed in three different models of experimental colitis. Moreover treatment with diallyl disulfide, an $H_2S$-donating molecule, normalized levels of $H_2S$ synthesis and significantly reduced the disease severity in the rats with hyperhomocysteinemia [68].

Further mechanisms that may contribute to the beneficial effects of $H_2S$ in promoting GI mucosal integrity and repair include the recently demonstrated stimulatory effects of this mediator on gastric bicarbonate secretion [83], and on mucus secretion [84]. The latter can promote a stronger barrier function of the GI mucosa, thereby limiting exposure to luminal bacteria [84]. Suppression of colonic $H_2S$ synthesis in mice with colitis markedly reduced mucus granule formation in the colon, in parallel with an exacerbation of the disease. $H_2S$ has also been shown to have direct inhibitory effects on the growth of different bacterial strains in the lumen of the gut [84]. Together these effects of $H_2S$ limit exposure of inflamed sites in the GI tract to luminal bacteria and may in part explain the pro-resolution effects of $H_2S$.

### 3.4. $H_2S$-based anti-inflammatory therapeutics

As was the case for NO, soon after reports of the physiological importance of $H_2S$ had been reported, several groups began to try to develop novel therapeutics based on release of $H_2S$ or inhibition of its synthesis [85–88]. Below is a summary of some of the drugs designed to target inflammatory processes.

#### 3.4.1. Inflammatory bowel disease

The incidence of IBD has been increasing steadily over the past 5 decades, and the pathogenesis of this group of disorders (which includes Crohn's disease and ulcerative colitis) remains poorly understood. Biological therapies targeting cytokines such as TNFα a have been very commercially successful over the past 20 years, but they are expensive, can trigger significant adverse events and have a high failure rate. Thus, there is still a need for safe, affordable medications for reducing inflammation and promoting repair of tissue injury. Mesalamine (5-aminosalicylic acid) is the first-line therapy for IBD. It is the active component of sulfasalazine, which was developed for treatment of arthritis, then found to be effective in patients with IBD. Mesalamine is a relatively weak anti-inflammatory drug, so high doses have to be administered, and typically in formulations that prevent absorption in the upper GI tract. On the other hand, mesalamine is very safe, with a very low incidence of largely mile adverse effects. While sometimes referred to as an NSAID, mesalamine actually has very weak inhibitory activity on COX, and that activity is very unlikely to contribute to its beneficial effects in patients with IBD. Its anti-oxidant actions have been suggested to account for most to its beneficial effects [89].

A drug developed by Antibe Therapeutics, ATB-429, is a derivative of mesalamine that releases $H_2S$. It exhibits significantly enhanced anti-inflammatory, pro-resolution and pro-healing effects in rodent models of colitis [66]. It has been shown to be very effective when given orally or intrarectally, with negligible absorption of the drug itself, or of mesalamine liberated from the drug. The low level of absorption of ATB-429 raises the possibility of it being used to treat inflammatory conditions throughout the GI tract, with no need for the types of formulations that have been employed in commercial preparations of mesalamine to prevent its absorption. Independent of the benefits associated with reduced absorption, ATB-429 has been shown to be significantly more potent than mesalamine at reducing GI inflammation in rat and mouse models of colitis [66]. Moreover, ATB-429 produced anti-inflammatory actions not seen with equimolar doses of mesalamine, including inhibition of tissue expression of a number of pro-inflammatory cytokines and chemokines (IL-1, IL-8, IL-12, TNFα, RANTES), but sparing of the expression of IL-10 [66]. Unlike mesalamine, ATB-429 also exhibited significant anti-nociceptive effects in a rat model of visceral pain [90].

#### 3.4.2. Acute and chronic pain

Among the most commonly used classes of drugs are NSAIDs: nonsteroidal anti-inflammatory drugs. They are used on a chronic basis by hundreds of millions of people suffering from disorders such as osteoarthritis, rheumatoid arthritis and ankylosing spondylitis. They are widely used an acute basis for treatment of a range of disorders characterized by severe pain (e.g., sports injuries, dental pain, gout, dysmenorrhea, post-surgical trauma, etc.). The greatest limitation to the use of this class of drugs is their propensity to cause ulceration and bleeding in the GI tract [91]. This carries an enormous economic burden to health care systems, particularly when the injury occurs in the distal small intestine, where it is difficult to detect, and for which no preventative or curative treatments are available [92,93]. $H_2S$-releasing NSAIDs have been developed by a number of groups, some of which are focusing primarily on their use as anti-inflammatory and analgesic agents, and some of which are focusing on their potential utility as chemopreventative agents for various types of cancer and/or for neurodegenerative diseases. The major advantage of the $H_2S$-releasing NSAIDs is their greatly reduced propensity to cause ulceration and bleeding in the GI tract [50,75,94–97]. Extensive pre-clinical studies demonstrate that $H_2S$-NSAIDs are at least as effective and potent as the parent NSAIDs in models of inflammation/pain [50,75,94–96]. These drugs also inhibit the activity of COX-1 and COX-2 with similar potency as the parent NSAIDs [50,75,94–96].

The GI safety of $H_2S$-NSAIDs has been extensively studied. In healthy rats, ATB-346 was found to produce negligible hemorrhagic damage in the stomach, while naproxen caused extensive injury [75]. With repeated dosing over several days, naproxen induces extensive ulceration and bleeding in the small intestine. However, no damage was observed when ATB-346 was administered in the same manner at equimolar doses [75]. Greatly enhanced GI safety was also observed in animal models that attempted to mimic the clinical scenario, where patients with arthritis have co-morbities (obesity, diabetes, old age, concomitant use of anti-coagulants, etc.) that substantially increases their risk of developing significant GI bleeding. In all such models, ATB-346 spared the animal of ulceration and bleeding, while naproxen caused significant damage [75,94].

## 4. Carbon monoxide

CO is produced via the enzyme heme oxygenase, which exists as constitutive (HO-2) and an inducible (HO-1) isoforms. The latter enzyme acts as a sensor of cellular stress, and the CO it generates can limit tissue injury [98]. Approaches to exploit these beneficial actions of CO in a clinical setting include the use of CO as an inhaled

gas and of CO-releasing molecules (often referred to as "CO-RMs" or "CORMs"), which can be administered orally. There have also been attempts to develop small molecules that can up-regulate HO-1 expression, thereby facilitating more CO production. Like NO and $H_2S$, CO exhibits cell and tissue protection through anti-apoptotic, anti-inflammatory, and anti-proliferative effects [98]. Other than the known interaction of CO with transition metals, the molecular targets of CO are largely unknown [2], though it has been suggested that ion channels are likely targets [99].

CO-RMs have been shown to exert powerful cytoprotective and anti-inflammatory actions, likely attributable in part to their effects on metal carbonyls that can influence oxidative stress, redox signaling and cellular respiration [98]. These molecules have been shown to be effective in a wide range of animal models, including pancreatitis, hepatic ischemia-reperfusion, colitis, cutaneous wound healing, neuropathic pain and osteoarthritis [98]. Like NO and $H_2S$, CO can suppress leukocyte adherence to the vascular endothelium and reduce pro-inflammatory cytokine expression by inhibiting NF-κB [100–103]. Also in line with the actions of the other two major gaseous mediators, CO has been shown to exert particularly potent protective effects in the GI tract [104].

Inhalation of CO is being evaluated as a means to reduce inflammation. Chiang et al. [105] reported that inhaled CO markedly reduced neutrophil infiltration into the peritoneum of mice, and increased heme oxygenase activity. They also showed a dramatic shift in the production of pro-resolving mediators: increased production of resolvin $D_1$ and maresin 1, and decreased production of leukotriene $B_4$. The phagocytic activity of macrophages was also significantly enhanced by treatment with CO. This work was extended to a model of bacterial pneumonia in baboons by Dalli et al. [106]. They observed that the plasma and leukocyte lipid mediator profiles in baboons with pneumonia were significantly shifted, with a reduction in the pro-resolution phenotype. However, this could be partially reversed by inhalation of CO. CO has shown therapeutic potential in animal models of acute lung injury, including those involving endotoxin challenge, oxidative lung injury, ischemia-reperfusion injury, pulmonary fibrosis, ventilator-induced lung injury, and lung transplantation [98,107]. Inhaled CO is presently being assessed in clinical trials to determine if it can improve lung function and survival in a critical care setting.

## 5. Future directions

The discovery that a gas such as NO could stimulate an enzyme and contribute to the body homeostasis triggered a re-thinking of many biology concepts. As described in this review, another two gasotransmitters, CO and $H_2S$, have been discovered that also contribute to several pathophysiological processes. In particular, $H_2S$ has been shown to share many features with NO. These gases can contribute to several different phases of resolution of inflammation as summarized in this review of the relevant literature. However, still there are several issues that need to be clarified through more detailed and focused studies. Increasing the knowledge of how these gases contribute to the resolution of inflammation and getting more insights into the cross-talk among them will clarify the molecular basis of resolution in different inflammatory-based diseases allowing the development of new therapeutic approaches.

## Disclosures

Drs. Wallace and Cirino are founders of Antibe Therapeutics Inc., which is developing hydrogen sulfide-releasing drugs.

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